Published in final edited form as:

Ann N Y Acad Sci. 2010 March ; 1192(1): 410–421. doi:10.1111/j.1749-6632.2009.05239.x.

Role of the polycystin-primary cilia complex in bone development and mechanosensing

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Abstract

Pkd1 encodes PC1, a transmembrane receptor-like protein, and *Pkd2* encodes PC2, a calcium channel, which interact to form functional polycystin complexes that are widely expressed in many tissues and cell types. The study of autosomal dominant polycystic kidney disease (ADPKD), caused by inactivating mutations of PKD1 or PKD2 genes, has elucidated the functions of polycystins and their interdependence on primary cilia in renal epithelial cells. We have found that Pkd1 and Pkd2, as well as primary cilia, are present in osteoblasts and osteocytes. In addition, we have found that loss of polycystin-1 (Pkd1) function in mice results in abnormal bone development and osteopenia due to the impaired differentiation of osteoblasts. It is likely that the polycystin/primary cilia complex responds to a multitude of environmental clues affecting skeletal development and bone formation postnatally. Overall, polycystins in bone may define a new target for developing anabolic agents to treat osteoporotic disorders.

Keywords

polycystin-1; Runx2; osteoblasts; osteocytes; mechanosensing; Kif3a; development

Polycystins (PC1 and PC2) and primary cilia, which are expressed in the osteoblast lineage, including preosteoblast, osteoblasts, and osteocytes,¹ form a polycystin-primary cilia signaling complex that may regulate skeletogenesis and function as a primary mechanosensor in bone.^{2,3}

Overview of polycystins/primary cilia signaling complex

The hypothesis outlined here has its origins in the study of the molecular and genetic basis for renal cystic diseases, which uncovered the developmental and mechanosensing functions of polycystins and primary cilia in renal epithelial cells. Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in the *PKD1* gene (85% of families) or in the *PKD2* gene (10–15% of families).⁴ The PKD1 gene product, PC1, is a 4,303-amino acid cell-surface-expressed protein that has a multidomain extracellular region, an 11 transmembrane region, and an ~200-amino acid cytoplasmic C-terminal tail.⁵ PC1 is conserved across species; the predicted PC1 proteins in mouse and humans are approximately 79% identical. PKD2 encodes a receptor-activated calcium channel.⁶ PC1 and PC2 form heterodimers (a polycystin complex) that co-localize to the primary cilium via interactions between the C-terminus of PC2 and the C-terminal region of Kif3a,⁶ an intraflagellar transport protein that maintains primary cilia,^{7,8} which are nonmotile

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Conflicts of interest

The authors declare no conflicts of interest.

organelles with no central tubules (9 + 0 cilia). Polycystic kidney disease can also be caused by loss-of-function mutations in proteins required for cilia formation or function, including TG737, Kif3a, fibrocystin, and cystin,⁹ suggesting that the functions of polycystins and primary cilia are interdependent in the kidney.

The multiplicity of ways to activate PC1 and the diversity of coupled signal transduction pathways places this receptor-like protein in the unique position of sensing and translating changing environmental cues. PC1 potentially acts as a mechanosensor, a chemosensor, or as a sensor of cell-cell or cell-matrix interactions. Of these, its role as a mechanosensor has strong support, possibly representing an ancient evolutionarily conserved paradigm as evidenced by the presence of PC1 and PC2 homologues in *C. elegans* in male-specific sensory cilia involved in male mating behaviors.⁸ Bending of primary cilia on renal epithelial cells, either directly or in response to fluid flow, has been shown to cause an increase in intracellular calcium through the polycystin-protein complex,^{8,10} and this response is absent with mutations in PKD1 or PKD2 which do not activate flow-induced Ca²⁺ signaling. PC1 can also interact with itself via the Ig-like domains, bind to carbohydrates via its C-type lectin domains, and interact with extracellular matrix proteins and annexins via its leucine-rich repeats. Recent studies show calcium dependence of galactose binding to the C-type lectin domain, supporting a chemosensor role. PC1 also localizes to cell-cell junctions, where it could modulate adhesion of adjacent cells and also binds with $\alpha\beta$ 1-integrin and co-localizes to focal areas of cell membrane contact with type I collagen matrix,¹¹ thereby mediating cell-matrix interactions. Cleavage of PC1 at the G protein-coupled receptor proteolytic site (GPS) upstream of the first TM segment may be required for bioactivity.¹²

The PC1 C-terminus serves as a scaffold for the regulation of a variety of signal transduction pathways. Co-assembly of PC1 and PC2 forms a Ca²⁺-permeable mechanosensitive ion channel in renal epithelial cells. In addition, the PC1 C-terminus also interacts with paxillin, Fak, and tuberin and activates G proteins and ERK. A C-terminus cleavage product interacts with P100/STAT6 to regulate gene transcription¹³ and couples with calcineurin/NFAT, RGS7/14-3-3, PKC- α , cJun-NH2-terminal kinase-mediated AP-1 transcription and the JAK-STAT-P21/Chi-1 signaling pathways.¹⁴ PC1 is also coupled to Wnt signaling,¹⁵ but there are inconsistent reports in the literature regarding whether PC1 activates Wnts through the canonical pathway or inhibits Wnt signaling through the noncanonical pathway.^{16,17} PC1 and β -catenin are present in a multiprotein plasma membrane complex and at sites of cell-cell contact. PC1-CT is reported to positively modulate the Wnt signaling pathway via inhibition of glycogen synthase kinase-3 β , stabilization of β -catenin and stimulation of TCF-dependent gene transcription. In contrast, the soluble cleaved C-terminal tail of PC1 is reported to bind to β -catenin in the nucleus and inhibit its activity.¹⁷

The pathologic functions of polycystin/primary cilia in the kidney are better understood than their physiologic functions. Indeed, disruption of polycystins or primary cilia complex in the kidney results in a common phenotype characterized by elevated rates of proliferation and apoptosis and increased fluid secretion in renal epithelial cell cysts. A multiplicity of physiological functions have been proposed, including the maintenance of the differentiated state, regulation of renal tubular morphology, regulation of cell proliferation in a tumor-suppressor-like fashion, regulation of planar cell polarity, and apoptosis/survival.¹⁸

There is strong support for extrarenal functions of the primary cilium/polycystin complex. Primary cilia and polycystins are ubiquitously expressed in adult and embryonic tissues. PC1 is highly expressed in the kidney and developing mesonephros, as well as in neural tissue, cardiomyocytes, endodermal derivatives,^{19,20} and bone.² Also, ADPKD is a multi-system disorder involving formation of cysts in the kidney and extrarenal tissues, including

intracranial and aortic aneurysms, and cystic disease of liver and pancreas. There are several mouse models with deletion/mutations of *Pkd1* that support essential functions of PC1 in multiple tissues, including kidney, pancreas, liver, lung, placenta, cardiovascular (both endothelium and smooth muscle, involving blood vessels and heart), and bone (including defects in osteoblast and chondrocyte development and/or function).^{21–23} In general, *Pkd1* mutant mouse models typically die prior to 16.5 dpc. and it has been difficult to determine which of the extrarenal manifestations are due to direct functions of polycystins in affected organs or indirectly caused by systemic effects related to loss of function in other organs.

Obstacles to understanding polycystin/primary cilia function in bone

The potential ability to integrate a variety of environmental cues into multiple signal transduction pathways that are known to regulate skeletal development makes the polycystin/primary cilia complex an attractive regulatory target in bone. Why, then, have polycystins and primary cilia received so little attention in skeletal biology? First, there is controversy regarding whether primary cilia and polycystin complexes are actually present in osteoblasts. In spite of the fact that Federman and Nichols documented the presence of primary cilia in osteocytes by electron microscopy more than 20 years ago,²⁴ this observation did not receive wide acceptance. The presence of cilia and their associated polycystin gene products have recently been reexamined using several complementary methods, and there is unequivocal evidence for the presence of both primary cilia and polycystins in osteoblasts/osteocytes.¹ In particular, MC3T3-E1 osteoblasts and MLO-Y4 osteocytes express PKD1 and PKD2 as well as cilia-associated transcripts Tg737 and Kif3a cilia genes (Fig. 1A and B). In addition, immunohistochemical analysis using an antibody to β -tubulin and SEM detected primary cilia-like structures in MC3T3-E1 osteoblasts and MLO-Y4 osteocytes. These findings in cell culture were confirmed by whole-mount immunostaining of 12-day-old mouse calvaria, which showed that primary cilia are present on the calvarial surface of osteoblasts and primary osteocytes localized within lacunae (Fig. 1C). *Pkd1* is also expressed in the perichondrium of vertebrae and long bones and expressed between E12.5 and E17.5 in neural crest cells and sclerotic mesenchyme, which give rise to intramembranous and endochondral bone.

Second, the lack of a demonstrable bone phenotype in patients with ADPKD delayed the investigation of PKD1 function in bone. The apparent lack of abnormalities in other tissues expressing PC1 has several potential explanations.²⁵ The molecular genetics/pathogenesis of ADPKD is complex, since the kidney disease is molecularly recessive, requiring tissue-selective inactivation of the other PKD1 allele at the cellular level by a “two-hit” mechanism to manifest renal cystic disease.^{26,27} The recessive nature of ADPKD disease and the requisite for a second hit may explain the paradox of why other organs, such as bone, that express PKD1 are not affected in patients with ADPKD, but exhibit severe abnormalities in homozygous mutant mouse models lacking both functional alleles. Indeed, other organ involvement, including skeletal abnormalities, might be expected in humans with global homozygous mutations, which are embryonically lethal. In addition, loss of PKD1 in a few renal cells are sufficient to cause cystic lesions that affect adjacent normal renal tissues, whereas the loss of PKD1 in only a few osteoblasts or osteocytes would be expected to be clinically unapparent. Excessive PTH may interact with the *Pkd1* mutation in osteoblasts to fundamentally alter or mask the underlying bone phenotype in ADPKD patients with superimposed CKD. In this regard, a feature of ADPKD renal cells is a phenotypic switch that reverses the typical antiproliferative effects of cAMP to a proliferative effect.²⁸ If a similar switch occurs in osteoblasts, the effects of PTH, which can be an anabolic agent in bone, might be accentuated, leading to increments in bone mass that counter the primary osteopenia found in the absence of PTH stimulation. Involvement of other tissues may also have been overlooked. Recent CT scan studies of lungs of ADPKD patients show a three-

fold increase in the prevalence of bronchiectasis compared to controls.²⁹ Clinical data regarding BMD measurements controlled for age, sex, renal failure, and degree of secondary hyper-parathyroidism in ADPKD, which would be needed to uncover small reduction in BMD in the heterozygous state, are not available.

Evidence that PC1 regulates skeletogenesis

At present the major evidence supporting a role of polycystin/primary cilia in bone is derived from the study of mutant mouse models. Analysis of the skeletal phenotype and osteoblasts derived from a variety of *Pkd1*-deficient mouse models supports a role of PC1 in bone development as well as a primary role in osteoblast function. The skeletal abnormalities have been best described in the *Pkd1*^{m1Bei}-null mouse (*Pkd1*^{m1Bei/m1Bei}), which has a point mutation (T to G at 9248 bp) causing an M to R substitution affecting the first transmembrane domain of Pkd1. Homozygous *Pkd1*^{m1Bei/m1Bei} mice are embryonically lethal and have a kidney phenotype similar to that observed in *Pkd1* knockout mice,²⁰ but heterozygous mice have no demonstrable kidney phenotype and normal survival. Cartilage formation was normal in E13.5 wild-type (WT, *Pkd1*^{+/+}) and *Pkd1*^{m1Bei/m1Bei} embryos, but in E14.5 and E15.5 *Pkd1*^{m1Bei/m1Bei} embryos there were reduced amounts of mineralized bone (Fig. 2A). Defects were observed in both the calvaria and long bone, suggesting that both intramembranous and endochondral bone formation was affected. Histologic analysis confirmed the delay in endochondral ossification in long bones of E15.5 *Pkd1*^{m1Bei/m1Bei} embryos (Fig. 2B). Homozygous deficient mice were also characterized by delay in vascular invasion, resulting in the absence of a bone marrow cavity. These mice also exhibited a narrow bone collar. Loss of one functional *Pkd1* allele in the adult resulted in low bone mineral density (BMD) in 12-week-old *Pkd1*^{m1Bei} heterozygous mice (*Pkd1*^{+/m1Bei}) (Fig. 2C). Heterozygous *Pkd1*^{+/m1Bei} mice have a 10% reduction BMD. μ CT analysis revealed that the osteopenia in heterozygous *Pkd1*^{+/m1Bei} mice was caused by a reduction in trabecular bone volume (33%) and cortical bone thickness (6%) (Fig. 2D). In addition, histologic analysis of bone identified a significant decrease in mineral apposition rate in 12-week-old *Pkd1*^{+/m1Bei} mice compared with age-matched *Pkd1*^{+/+} mice, suggesting impaired osteoblast-mediated bone formation (Fig. 2E). These changes are not due to impaired renal function, since serum creatinine was normal in 12-week-old *Pkd1*^{+/m1Bei} mice and these mice did not have evidence of renal cysts. Further evidence for osteoblast dysfunction includes a reduction in *osteocalcin*, *osteoprotegerin*, and *Rank ligand* in serum from heterozygous *Pkd1*^{+/m1Bei} mice at 12 weeks of age. Serum level of TRAP, a marker of bone resorption, was also reduced in heterozygous *Pkd1*^{+/m1Bei} compared to WT littermates. *Runx2-II* expression, a master gene controlling osteoblast development,³⁰ was significantly diminished in heterozygous *Pkd1*^{+/m1Bei} mice at both E15.5 and 12 weeks of age. In addition, reduction in *Runx2-II* expression was *Pkd1* gene dose-dependent, with levels being less in *Pkd1*^{m1Bei/m1Bei}-null compared to *Pkd1*^{+/m1Bei} heterozygous embryos (Fig. 2F). Moreover, concomitant defects in downstream gene expression were observed in *Pkd1*^{m1Bei}-deficient mice, including a significant reduction in osteoblast (*osteocalcin*, *osterix*, *osteoprotegerin* (*OPG*), and *Rank ligand* (*RanKL*)) and osteoclast (*tartrate resistant acid phosphatase*, *TRAP*) specific transcripts in both embryos and adult mutant mice by real-time RT-PCR. These findings suggest that low bone formation rates rather than increased resorption contribute to the low BMD and bone volume of femurs observed in heterozygous *Pkd1*^{+/m1Bei} mice.

A functional linkage between PC1 and *Runx2-II* *in vivo* was investigated by crossing *Pkd1*^{+/m1Bei} mutant mice with *Runx2-II*-deficient mice to create double heterozygous mice lacking one functional allele of both *Runx2-II* and the *Pkd1* gene encoding PC1 (Fig. 2C–F). While either *Runx2-II*^{+/-} and *Pkd1*^{+/m1Bei} heterozygous mice had an approximate 10% reduction in BMD, consistent with our prior characterization of these mice, the combined

loss of one allele of *Runx2-II* and *Pkd1* resulted in additive effects to reduce BMD, as evidenced by the 22% reduction in BMD in the double heterozygous *Runx2-II^{+/-}/Pkd1^{+/-}* mice. μ CT analysis revealed that the reduction in bone mass in *Pkd1^{+/-}* and *Runx2-II^{+/-}* heterozygous mice was caused by a reduction in trabecular bone volume (43% and 50%, respectively) and cortical bone thickness (11% and 18%, respectively). Double heterozygous *Runx2-II^{+/-}/Pkd1^{+/-}* mice had greater loss in both trabecular (83%) and cortical bone (28%) than single heterozygous mice, consistent with additive effects of *Runx2* and *Pkd1* on bone formation (Fig. 2C and D). Histologic analysis of bone showed a significant decrease in mineral apposition rate in *Pkd1^{+/-}* and *Runx2-II^{+/-}* heterozygous mice compared with age-matched WT mice, and an even greater reduction in double heterozygous *Runx2-II^{+/-}/Pkd1^{+/-}* mice (Fig. 2E), suggesting additive effects to impair osteoblast-mediated bone formation. More importantly, *Runx2-II* expression was reduced in heterozygous *Pkd1^{+/-}* and to a greater degree in double *Runx2-II^{+/-}/Pkd1^{+/-}* mice (Fig. 2F). *Runx2-I* expression was not different from WT mice in either single or double heterozygous mouse models, consistent with a selective effect of *Pkd1* to regulate the p1-*Runx2-II* isoform.

Studies of osteoblasts derived from calvaria of E15.5 *Pkd1^{m1Bei/m1Bei}* mutant mice *ex vivo* suggest that the *in vivo* abnormalities are due to primary defects in osteoblast function (Fig. 3). *Pkd1^{m1Bei/m1Bei}* osteoblasts have defective differentiation *ex vivo* associated with selective reduction in *Runx2-II*. *Pkd1^{m1Bei/m1Bei}* osteoblasts failed to differentiate into mature osteoblasts, which was shown by lower ALP activity and mineralization of extracellular matrix as assessed by alizarin red S staining in *Pkd1^{m1Bei/m1Bei}* compared to WT osteoblast cultures (Fig. 3A–D). *Pkd1^{m1Bei/m1Bei}* osteoblasts also have lower basal intracellular calcium levels compared to WT osteoblasts (Fig. 3E), consistent with mutations of *Pkd1* affecting its coupling to *Pkd2*. Transfection of gain-of-function *Pkd1* C-terminal constructs with an intact coiled-coil domain (AT) stimulated *Runx2-II* promoter activity in both osteoblast and osteocytes (Fig. 4A). Significant increases of endogenous *Runx2* message expression as well as its downstream genes *osteocalcin*, *osterix*, *osteoprotegerin* and *Rank ligand* in MC3T3-E1 stably transfected with the PC1-AT construct containing the coiled-coil domain (Fig. 4B).

It is likely that polycystins regulate multiple signaling pathways in osteoblasts, but the best characterized is activation of intracellular calcium-dependent signaling pathways. There is evidence for dysfunctional intracellular calcium in PC1-null osteoblasts. PC1-AT overexpression increases intra-cellular calcium levels in osteoblasts (Fig. 4C). In addition, BAPTA, an intracellular calcium chelator, and thapsigargin, an ER Ca^{2+} -ATPase inhibitor that depletes calcium stores completely abolished PC-AT-stimulated *Runx2-II* promoter activity in MC3T3-E1 osteoblasts (Fig. 4D). Two composite NF1 and AP1 binding sites conserved across species are present in the proximal *Runx2* promoter (Fig. 4E). Further deletion analysis confirmed that each of the NF1 and AP1 sites independently account for PC1-mediated activity in MC3T3-E1 osteoblasts (Fig. 4F). NF1 isoforms are expressed in MC3T3E-1 osteoblasts but are not upregulated by PC1-AT (Fig. 4G). CHIP assay using anti-NF1 antibody, however, demonstrates Nfi-binding to sites between the –415 to –342 bp region of *Runx2* P1 promoter (Fig. 4H). Transient co-transfection *c-Jun* plasmid with *Runx2* P1–420-Luc (p0.42*Runx2*P1-Luc) also stimulated *Runx2* P1 promoter activity (Fig. 4I), indicating that AP-1 binding sites may be involved this activity. Together, these findings imply PC1 selective activation of *Runx2-II* isoform transcription through intracellular calcium and Nfi/AP1 pathways.²

Whether PC1 function in bone requires primary cilia is not known, but several observations support a role of primary cilia function in skeletal development. First, as noted above, cilia are present in osteoblasts, osteocytes and in certain chondrocytes (chick sternal hyaline and

articular chondrocytes).¹ There are also several diseases of primary cilium that have an associated bone phenotype. For example, deletion of *polaris* resulted in loss of cilia and abnormal development of the appendicular skeleton.³¹ Recent insights into the genetic basis of the oral-facial-digital (OFD) syndrome, which causes craniofacial and digital abnormalities and polycystic kidney disease, establish a potential link between bone abnormalities and primary cilia since the OFD1 gene encodes a ubiquitous protein found in the centrosome and the basal body in primary cilia.³² Bardet-Biedl syndrome (BBS), which is caused by BBS1–12 genes involved in primary cilia dysfunction, is associated with craniofacial abnormalities due to aberrant cranial neural crest cell migration during development. Recessive cystic kidney diseases caused by mutations in *NPHP6*, *MKS1*, and *MKS3* are also associated with bone changes. In mouse models, the conditional deletion of *polaris* in the developing limb disrupts endochondral bone formation.³¹ While implicating primary cilia in skeletogenesis, these findings do not necessarily support a role for polycystins, since primary cilia house other signaling molecules, such as *Shh* and *Ihh* pathways, which are affected by loss of cilia.

Because of these considerations, we propose the hypothesis that the polycystins/primary cilia function as a “hub” or a common connection point that permits cells in the osteoblast lineage to sense diverse environmental signals during skeletal development and translate these into multiple signaling pathways. Depending on the level of cell differentiation (e.g., mesenchymal precursor, osteoblasts, or osteocyte) and environmental signals (e.g., cell-cell, cell-matrix, flow, and/or chemokines), polycystins and primary cilia activate different signaling pathways and may also act as a “switching station” to balance the relative activation of calcium-dependent, non-canonical Wnt and canonical Wnt signaling pathways. If so, different stages of development within the osteoblast lineage and different environmental signals would determine the net effect of these pathways on osteoblastic proliferation, differentiation, and apoptosis.

Cilia-PC1/PC2 complex: a novel candidate for the primary bone mechanoreceptor?

Mechanical stimulation of the skeleton by exercise leads to a bone anabolic response due to increased osteoblastic proliferation and matrix deposition.³³ In contrast, the absence of mechanical stimulation, as occurs with immobilization, disuse, and exposure to low gravity, causes bone loss. Osteocytes are key mechanosensitive cells in bone that transduce mechanical forces into anabolic cell signals leading to new bone formation. Bone fluid flow shear stress is likely initiated through the back-and-forth pulsatile movement of extracellular fluid in the bone microenvironment during walking or running cycles. The theoretical basis for fluid-derived shear stress stimulation of bone cells is derived from the microstructural organization of the lacunar-canalicular microstructure and applies to osteocytes not osteoblasts.³⁴ Multiple, complementary and possibly redundant pathways have been identified in osteoblasts/osteocytes that are activated by mechanical forces, including several G protein-coupled receptors (e.g., prostaglandin and purinergic receptors), integrin receptors, connexins/gap-junctions and hemichannels, and stretch-activated and purinergic channels, but the identity of the “primary mechanosensor” has not been unequivocally established. It is possible that the primary mechanosensing pathway found in the kidney, namely, the cilia-polycystin complex, is also responsible for mechanosensing in bone. It is possible that interstitial fluid flow through the pericellular space of the lacunocanalicular system could bend, deform/stretch, or provide chemical stimuli to a single cilium that projects from the body of the osteocyte. Published evidence for this possibility is much less than for the role of cilia/polycystins in bone development, but many of the mechanically activated intracellular pathways involved in the anabolic bone responses are also activated by the cilia-PC1/PC2 complex. The chemical ablation of primary cilia in osteoblast cultures

results in attenuated responses to dynamic fluid flow.³⁵ The most compelling published data at present show that mice with a conditional deficiency for PC1 in neural crest cells, osteoblasts, and chondrocytes have impaired anabolic response to a load induced by midpalatal suture expansion.^{36,37}

Future studies

To further examine the skeletal functions of polycystins, it will be necessary to use osteoblast lineage-specific promoter-Cre mice to selectively delete *Pkd1* and *Pkd2* from different stages within the osteoblast lineage, including preosteoblasts, mature osteoblasts, and osteocytes. To explore potential interdependence of polycystin and primary cilia in bone, the effect of bone-specific deletion of *Kif3a* to disrupt primary cilia formation in the osteoblast lineage can be compared to that of osteoblast-specific deletion of *Pkd1* and *Pkd2*. Finally, to understand the cellular mechanisms underlying the osteopenia in mutant mice, the effects of polycystins on osteoblast proliferation, differentiation, and apoptosis need to be further investigated *in vitro*.

Pharmacologic induction of bone formation through anabolic actions of polycystins?

Osteoporosis is caused by an imbalance between bone formation and resorption, leading to low bone mass and fractures. Osteoclasts are important targets for antiresorptive therapies, which have been the mainstay of osteoporosis treatment.³⁸ Although effective in reducing fractures, antiresorptive therapies have limited ability to restore trabecular architecture and do not address the deficits in osteoblast-mediated bone formation and inefficient osteoblast recruitment that are also important in the pathogenesis of osteoporosis. There is a paucity of anabolic targets in the osteoblastic lineage to generate new osseous structures. The only anabolic agent currently available is recombinant human parathyroid hormone (PTH-1–34 or 1–84), which targets their G protein-coupled receptor.³⁹ Efforts are under way to develop new anabolic agents by targeting the Wnt/ β -catenin pathway with antibodies to sclerostin (Sost) and dickkopf-1 (Dkk1) or activin receptor fusion protein that functions as an activin antagonist. Polycystins have the potential to uniquely target both G-protein and Wnt-signaling pathways, thereby encompassing two of the known anabolic pathways in bone. Ongoing studies that examine the effect of conditional deletion of polycystins and components of primary cilia in preosteoblasts, osteoblasts, and osteocytes, however, are necessary proof-of-concept studies to define the anabolic potential of polycystins and primary cilia in skeletogenesis and mature bone function. In the future, such knowledge may be exploited to develop “ligand” activators for polycystins as novel anabolic agents for stimulating osteoblastic function. Such agents may be useful for treating osteoporosis as well as pharmacologically loading the skeleton which would be useful in management of bone loss due to immobilization and weightlessness in space travel.

Acknowledgments

This work was supported by the Grants R01-AR049712 and R21-AR056794 from the National Institutes of Health.

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Figure 1.

Evidence for polycystins and primary cilia in osteoblasts and osteocytes. (A) Immunostaining with α -tubulin antibody in MC3T3-E1 osteoblasts and MLO-Y4 osteocytes showing a single primary cilium projecting from the cell surface (*arrow*). (B) Cilia genes, polaris (Tg737) and Kif3a, and polycystins, Pkd1 and Pkd2, are expressed in MC3T3-E1 osteoblasts and MLO-Y4 osteocytes by RT-PCR. (C) Whole-calvaria immunostaining for primary cilia in osteocytes (*top*) and osteoblasts (*bottom*) from 12-day-old Dmp1-GFP mice. GFP-positive osteocytes, GFP-negative osteoblasts, and blue DAPI-stained nuclei. *Arrows* show single cilium per cell.

Figure 2.

Defective skeletogenesis and abnormal gene expression in homozygous *Pkd1*^{m1Bei/m1Bei} embryos. (A) Alizarin red/alcian blue whole-mount of wild-type (WT) and *Pkd1* mutant embryos. Red is calcified bone, and blue is unmineralized cartilage. The skeletal calcification was markedly delayed in E14.5-E15.5 *Pkd1*^{m1Bei/m1Bei} embryos as indicated by arrows. (B) Histologic analysis (H&E staining) of endochondral ossification in longitudinal sections of decalcified femurs of E15.5 WT and *Pkd1* mutant embryos. Low magnification (40×) of entire femur (left), higher-power magnification (100×) of metaphyseal and diaphyseal regions (right). Arrow bar indicates bone marrow cavity (BMC) and arrows indicate bone collar (BC). (C–F) Effects of combined *Pkd1* and *Runx2-II* deficiency on BMD (C), μCT (D), bone formation rate (E), and *Runx2-II* expression (F). The effects of heterozygous *Runx2-II* and *Pkd1* deficiency are additive. Data are the mean ± SEM from at least three individual samples. * indicates significant from wild-type and # indicates significant from either *Pkd1*^{+/-}m1Bei or *Runx2-II*^{+/-} heterozygous mice at *P* < 0.05 by one-way analysis of variance.

Figure 3. *Pkd1*^{m1Bei/m1Bei} osteoblasts have a developmental defect *ex vivo*. (A) Runx2 expression in immortalized *Pkd1*^{m1Bei/m1Bei} osteoblasts. (B) Histochemical staining of mineralization nodules. *Upper panel* depicts representative 6-well plates derived from 14-day-old wild-type (WT) and *Pkd1*^{m1Bei/m1Bei} osteoblasts stained for mineralized nodules with alizarin red-S. *Lower panel* is a high-power (100×) magnification, showing well-defined mineralization nodules in WT osteoblasts and indiscrete foci of stain in *Pkd1*^{m1Bei/m1Bei} osteoblasts. (C) Quantification of mineralization. Alizarin red-S stain was extracted with 10% cetylpyridinium chloride and quantified. *PKD1*^{m1Bei/m1Bei} osteoblasts had time-dependent increments in alizarin red-S accumulation during 14 days of culture, but the accumulation was significantly lower at different time points compared with *Pkd1*^{+/+} osteoblasts. (D) ALP activity. *Pkd1*^{m1Bei/m1Bei} osteoblasts displayed time-dependent increments in ALP activities during 14 days of culture, but the ALP activity was significantly lower at different time points compared with *Pkd1*^{+/+} osteoblasts. (E) Steady-state intracellular calcium levels were lower in *PKD1*^{m1Bei/m1Bei} compared to WT mice.

Figure 4.

Signal transduction pathways linking Pkd1 to Runx2 in MC3T3-E1 osteoblasts. (A) Overexpression of PC1-AT stimulates p1, but not p2 promoter activity. (B) Effects of PC1-AT overexpression on osteocalcin, osterix, osteoprotegerin, and Rank ligand message expression in MC3T3-E1 osteoblasts. (C) Increased $[Ca^{2+}]_i$ in osteoblasts stably transfected with PC1-AT. (D) BAPTA and thapsigargin inhibit PC1-mediated stimulation of P1 promoter activity. Values sharing the same superscript are not significantly different at $P < 0.05$. * indicates significant difference from the slgΔ control at $P < 0.05$ by one-way analysis of variance. (E) NFI and AP1 binding sites are conserved across species between -415 to -342 region of *Runx2*-II P1 promoter. (F) Deletion analysis confirmed that each of the combined NFI and AP1 sites contributed to ~ 50% of the PC1-mediated activity in MC3T3-E1 osteoblasts. (G) NFIA, B, C, and X isoforms are all expressed in MC3T3-E1 osteoblasts. (H) ChIP assay confirmed NFI-binding sites between -415 to -342 bp region. (I) Transient co-transfection *c-Jun* plasmid with *Runx2* P1-420-Luc (p0.42*Runx2*P1-Luc) stimulated *Runx2* P1 promoter activity, indicating AP-1 binding sites may be involved this activity.